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Review

From Synapse to Nucleus: Calcium-Dependent Gene Transcription in the Control of Synapse Development and Function

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One of the unique characteristics of higher organisms is their ability to learn and adapt to changes in their environment. This plasticity is largely a result of the brain's ability to convert transient stimuli into long-lasting alterations in neuronal structure and function. This process is complex and involves changes in receptor trafficking, local mRNA translation, protein turnover, and new gene synthesis. Here, we review how neuronal activity triggers calcium-dependent gene expression to regulate synapse development, maturation, and refinement. Interestingly, many components of the activity-dependent gene expression program are mutated in human cognitive disorders, which suggest that this program is essential for proper brain development and function.

Introduction

Mammalian nervous system development occurs through an intricate genetic program that ensures that the diverse types of neurons and glia that make up the brain are generated in the correct number and migrate to the appropriate place by the time of the birth of the organism. However, sensory, cognitive, and motor experiences also play a key role in shaping neuronal circuitry by affecting the development and maturation of synaptic connections during postnatal nervous system development. Later in life, sensory experiences also affect synaptic function leading to the formation of long-lasting memories and alterations in the behavior of adult organisms. The importance of environmental cues for the development of the nervous system was demonstrated by the elegant work of Hubel and Wiesel in the visual system, which revealed that experience plays a critical role in shaping neuronal connectivity (Wiesel, 1982).

The effects of the environment on brain development are manifested through the release of neurotransmitters at specific synapses, which, following binding to appropriate receptors on the postsynaptic neuron, induce a number of biochemical signaling events in the postsynaptic neuron. One of the most prominent of these signaling events is a rapid and transient rise in calcium levels within the postsynaptic specialization. This local increase in calcium concentration can, depending on the cellular context, result in a number of short-term and long-term synapse-specific alterations, including the insertion or removal of glutamate receptor subunits from the membrane, the alteration of synaptic protein function via posttranslational modifications such as phosphorylation, and the stimulation of the translation or degradation of proteins at the synapse, which together lead to changes in synaptic function (reviewed in this issue of *Neuron*: Catterall and Few, 2008; Higley and Sabatini, 2008; Wayman et al., 2008b).

In addition to these local changes at the synapse, calcium influx into the postsynaptic neuron initiates a cascade of signaling events that culminates in the nucleus and results in the activation of a program of gene expression that promotes dendritic growth, synapse development, and neuronal plasticity. In this review, we will begin by describing initial studies revealing that calcium signaling in neurons induces gene transcription within the nucleus. We will discuss how an increase in intracellular calcium concentration is interpreted by the neuron and how this signal is propagated to the nucleus to induce the transcription of specific genes. We will then review how the newly synthesized mRNAs and proteins act within the neuron to affect synaptic development and plasticity. Finally, we will conclude with a discussion of how this program of activity-dependent gene transcription is important for organismal behavior and how mutation of components of this genetic program result in human cognitive disorders, including autism spectrum disorders.

Discovery that Transcription Can Be Induced by Neuronal Activity

The first clue that there might be genes whose expression is regulated by neuronal activity came from the observation that agonists of the nicotinic acetylcholine receptor, and other agents that induce membrane depolarization and the subsequent influx of calcium into cells through L-type VSCCs, trigger the rapid and transient activation of the *c-fos* proto-oncogene in neuronal cell lines (Greenberg et al., 1986). It was later shown that a variety of stimuli that mimic the effects of neuronal activity, such as bath application of neurotransmitters or membrane depolarizing levels of potassium chloride, also robustly induce *c-fos* transcription in primary neuronal cultures and that *c-fos* transcription also occurs in the intact brain in response to a wide variety of physiological stimuli (e.g., novel, visual, and social experiences, circadian entrainment, fear conditioning, and exposure to drugs of abuse) (Anokhin et al., 1991; Dragunow and Robertson, 1987; Hope et al., 1992; Morgan et al., 1987; Rusak et al., 1990).

Subsequent studies revealed that *c-fos* is one of several hundred genes that are regulated by neuronal activity as synapses are forming and maturing (Altar et al., 2004; Hong et al., 2004; Li et al., 2004; Nedivi et al., 1993; Park et al., 2006). Many of the activity-regulated genes like *c-fos* encode transcription factors that are believed to mediate the cellular response to synaptic stimulation by activating target genes that play important roles in cell survival, dendritic and axonal growth, synaptic development, and neuronal plasticity. In addition, many of the genes whose expression is directly regulated by neuronal activity encode proteins that act specifically at synapses to control various aspects of synaptic development and function. These include BDNF, a neurotrophin that regulates survival, dendritic growth, and excitatory and inhibitory synaptic development; Arc, a cytoplasmic protein that controls glutamate receptor endocytosis; Cpg15, a membrane-bound protein that regulates survival and dendritic growth; and Homer1a, which controls the formation of synaptic protein complexes, thus controlling synapse number (Cantallops et al., 2000; Chowdhury et al., 2006; Kang and Schuman, 1995; Korte et al., 1995; McAllister et al., 1995, 1997; Nedivi et al., 1998; Rial Verde et al., 2006; Sala et al., 2003). Because activity-regulated transcripts play such crucial and broad roles in neuronal development, considerable effort has gone into understanding the signaling mechanisms by which neurotransmitter release at synapses induces gene expression in the nucleus. Additional effort has been directed toward analyzing the function of activity-regulated genes in synaptic development and testing the hypothesis that these critical synaptic regulators, when mutated, are the cause of a wide range of neurological disorders.

Rises in Levels of Intracellular Calcium: The Route of Entry Matters

Like most excitable cells, neurons expend considerable energy in maintaining low basal levels of intracellular calcium by pumping calcium either into internal storage organelles or the extracellular space. By maintaining low levels of calcium within the cell, the neuron is able to respond rapidly and effectively to increases in intracellular calcium concentration. There are multiple ways in which cytoplasmic calcium levels can rapidly increase in the postsynaptic neuron: calcium can be released from intracellular stores maintained by the neuron, or extracellular calcium can enter the cytoplasm through voltage-gated calcium channels or either of two types of ligand-gated ion channels, the N-methyl-D-aspartate type (NMDA) or a-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type (AMPA) glutamate receptors (Berridge, 1998; Jonas and Burnashev, 1995). Interestingly, although each of these routes of calcium entry lead to significant increases in the concentration of intracellular calcium, depending on the mode of entry, there can be quite different responses in terms of gene induction (Bading et al., 1993; Ginty, 1997). For example, brain-derived neurotrophic factor (BDNF) is highly induced in excitatory neurons following calcium entry through L-type voltage-sensitive calcium channels (VSCCs) but much less effectively following calcium influx through NMDA receptors or other VSCCs, such as N-type calcium channels (Ghosh et al., 1994; Westenbroek et al., 1992). These and a variety of additional findings initially led to speculation that the route (i.e., the specific channel) through which calcium enters the neuron is critical for determining the pattern of gene induction. Recent studies have identified a variety of features of calcium channels that determine whether calcium influx through the channel will trigger a gene expression response. These include channel conductance properties, channel open times, subcellular localization of the channel, the ability of the channel to trigger an increase in the calcium concentration within the nucleus, and the association of the calcium channel with key signaling molecules (see below).

L-type VSCCs were the first of the neuronal calcium channels to be shown to be involved in the induction of activity-dependent gene transcription. L-type VSCCs have several properties that make them particularly well suited for conveying a calcium signal from the site of entry at the plasma membrane to the site of gene induction within the nucleus. For example, L-type VSCCs have a relatively slow inactivation rate and a high single-channel conductance for calcium, allowing these channels to flux the large amounts of calcium into neurons that is required to elicit a gene expression response (Gallin and Greenberg, 1995). In addition, L-type VSCCs are somatodendritically localized, enabling them to elevate calcium levels within the cell soma in close proximity to the nucleus, thus allowing efficient propagation of the calcium signal to the nucleus or elevating calcium levels directly within the nucleus (Catterall, 2000; Westenbroek et al., 1990). Furthermore, a number of signaling proteins important for gene induction associate with L-type VSCCs, including the protein kinase A anchoring protein (AKAP79/150), the tyrosine kinase Src, and the phosphatase calcineurin (Bence-Hanulec et al., 2000; Gray et al., 1998; Oliveria et al., 2007). AKAP79/150 recruits PKA to L-type VSCCs, leading to the phosphorylation of the channel, increasing its ability to flux calcium. At the same time AKAP79/150 recruits the phosphatase calcineurin to L-type VSCCs. Calcineurin activation is required for the translocation of the transcription factor NFATc4 to the nucleus and for NFATc4 activation as well as for the dephosphorylation and activation of MEF2 family transcription factors (Chin et al., 1998; Graef et al., 1999; Mao and Wiedmann, 1999). Additionally, it has been demonstrated that the calcium-sensing protein calmodulin binds to the intracellular domain of the L-type channel that, upon calcium influx, activates Ras/MAP kinase signaling to initiate gene transcription within the nucleus (Dolmetsch et al., 2001). Consistent with these observations, pharmacological blockade of L-type VSCCs as well as chelation of calcium in close proximity to the plasma membrane inhibits immediate-early gene induction (Murphy et al., 1991; Deisseroth et al., 1996).

Despite the obvious importance of calcium influx through L-type VSCCs for the induction of gene transcription in response to neuronal activity, calcium influx through other types of ion channels—especially during early development—also plays an important role in regulating gene expression. During early stages of synaptogenesis, the EphB family of receptor tyrosine kinases and their ligands the ephrins modulate NMDA receptor function to drive gene expression within the nucleus (Takasu et al., 2002). EphB receptors play important roles in synapse development and function, and during development, contact between postsynaptic EphB receptors and presynaptic ephrins may induce the association of NMDA receptors with EphB receptors (Dalva et al., 2000). Ephrin binding to EphBs also leads to the activation

of Src, a non-receptor tyrosine kinase that subsequently phosphorylates the NR2B subunit of the NMDA receptor, allowing for increased calcium influx through NMDA receptors upon release of the neurotransmitter glutamate at the developing synapse. While this calcium influx is sufficient to drive gene expression, future experiments will be required to determine the significance of these events during nervous system development.

Nonetheless, these data suggest a possible role for NMDA receptors in activity-mediated gene induction during development, and there is mounting evidence to suggest that complexes associated with NMDA receptors help mediate these transcriptional responses. NMDA receptors are heteromeric ion channels comprised of NR1 and NR2 subunits. Early in development, NMDA receptors are primarily composed of NR1 and NR2B subunits before undergoing a developmental switch that results in NR2B being replaced by NR2A (Carmignoto and Vicini, 1992). Upon binding glutamate NR2B subunit-containing NMDA receptors have longer durations of calcium influx than their NR2A-possessing counterparts, suggesting that NMDA receptors early in development are better suited to regulate gene expression in the nucleus. Consistent with this possibility, stimulation of NMDA receptors in young neurons triggers robust phosphorylation and activation of the cyclic-AMP response element binding protein, CREB, an important mediator of activity-dependent transcription, whereas stimulation of older neurons only induces the transient phosphorylation of CREB (Sala et al., 2000). Interestingly, a CREB phosphatase, protein phosphatase 1 (PP1), has been shown to physically associate with NMDA receptors in older neurons, possibly explaining why CREB phosphorylation and activation are so transient later in development (Westphal et al., 1999).

In addition to PP1, NMDA receptors interact directly or indirectly with a large number of signaling molecules (e.g., calmodulin, CaMKII, calcineurin, Tiam1, and proteins involved in Ras/MAP kinase signaling, including H-Ras, c-RAF1, Mek1 and -2, and Erk1 and -2), each of which has been implicated in the regulation of activity-dependent transcription (Husi et al., 2000; Tolias et al., 2005). Calmodulin, upon binding to calcium, undergoes a conformational switch enabling calmodulin to bind to and activate a number of effector proteins in the nucleus and the cytoplasm, including CaMKII and the phosphatase calcineurin (Chin and Means, 2000). Calcineurin dephosphorylates MEF2 transcription factors, a necessary step for their activation, while CaMKII phosphorylates and activates a number of activity-dependent transcription factors, including CREB and NeuroD. In addition to activating transcription factors directly, CaMKII phosphorylates the NMDA receptor-associated protein Tiam1, a guanine nucleotide exchange factor (GEF) that activates the small GTPase Rac1, a regulator of the actin cytoskeleton. Calcium influx through NMDA receptors also activates a number of other Rac GEFs, including betaPIX and Kalirin-7 (Xie et al., 2007; Saneyoshi et al., 2008). Actin in turn may regulate the serum response factor SRF by controlling the nuclear localization of its cofactor MAL (Vartiainen et al., 2007). SRF and CREB are also regulated by the Ras/MAP kinase pathway, which triggers RSK/MSK activation, leading to SRF phosphorylation at serine-103 and CREB phosphorylation at serine-133 (Heidenreich et al., 1999). Together these events promote activity-dependent gene activation and suggest an important role for NMDA receptors in regulating signaling to the nucleus to affect synapse development and maturation.

In addition to NMDA receptors, AMPA-type glutamate receptors (GluRs) are also able under some circumstances to activate gene transcription. AMPA receptors are tetramers comprised of various combinations of GluR1–4 subunits and mediate the majority of excitatory synaptic transmission in the central nervous system (CNS) (Derkach et al., 2007). The majority of AMPA receptors are not permeable to calcium. This is due to the fact that these channels contain GluR2 subunits that as a consequence of RNA editing have a positively charged arginine in the AMPA receptor's channel pore that renders the channel impermeable to calcium. However, calcium-impermeable AMPA receptors are able to promote activity-dependent transcription under conditions where glutamate binding to the AMPA receptor depolarizes the plasma membrane sufficiently to trigger opening of the L-VSCCs. In addition, calcium-permeable AMPA receptors (those lacking GluR2) can drive gene expression directly. In response to calcium influx through GluR2-lacking AMPA receptors, calcium/calmodulin promotes the activation of Ras-GRF (guanine nucleotide-releasing factor) proteins, which once activated stimulate Ras-ERK signaling pathways, which in turn trigger the phosphorylation of CREB at serine-133 and induce activity-dependent transcription (Tian and Feig, 2006). Interestingly, during early development, neurons preferentially express GluR2-lacking AMPA receptors (calcium permeable), while in the mature brain, GluR2-containing AMPA receptors (calcium impermeable) predominate, suggesting that during brain development different activity-dependent transcriptional programs may be induced than in the mature nervous system (Kumar et al., 2002).

The activation of glutamate receptors and L-type VSCCs leads to local increases in calcium near the mouth of these channels but can also trigger substantial increases in nuclear calcium. It is now appreciated that an elevation of calcium concentration within the nucleus is required in some cases for the induction of the activity-dependent gene expression program. For example, a rise in nuclear calcium is required for CREB-mediated, but not SRF-dependent, transcription, and blocking the activity-dependent elevation in nuclear calcium results in decreased neuronal survival and defective memory consolidation (Chawla et al., 1998; Hardingham et al., 1997; Limback-Stokin et al., 2004; Papadia et al., 2005).

How Calcium Signals within the Nucleus

As discussed above, calcium enters neurons through both somatodendritically and synaptically localized ion channels. Under circumstances where the calcium signal is propagated to the nucleus, it can trigger the activation of an intricate program of activity-dependent gene expression (Figure 1). Over 300 genes have been shown to be turned on and off again in response to neuronal activity, and remarkably, each gene has a unique time course and magnitude of induction (Lin et al., 2008). Elucidation of the mechanisms that keep these genes off until the right time and place and then allow their transient activation in response to neuronal activity has primarily come from the study of two

genes—the *c-fos* proto-oncogene and *Bdnf*. While *c-fos* can be activated by extracellular stimuli in a wide variety of tissues and cell types, *Bdnf* transcription is preferentially activated in response to neuronal activity and thus is induced primarily in neurons (West et al., 2001).

Like many activity-regulated genes, *Bdnf* has a complex genomic organization allowing for precise temporal, spatial, and stimulus-specific regulation of its transcription (Figure 2). The *Bdnf* gene is composed of at least eight discrete promoters that initiate the transcription of multiple distinct *Bdnf* mRNAs, each of which contains an alternative 5′ exon spliced to a common 3′ coding exon that employs either of two polyadenylation sites. The alternative promoters together with alternative splicing and polyadenylation result in the production of at least 18 distinct *Bdnf* mRNAs, each of which encodes an identical protein (Aid et al., 2007). Why the *Bdnf* genes give rise to so many mRNA transcripts, all of which encode an identical protein, is not yet fully appreciated, but it suggests that this organization may allow for tight temporal and spatial control of *Bdnf* transcription. Additionally, as these different mRNA species contain unique 3′ and 5′ untranslated regions (UTRs), it may be that sequences contained within the UTRs target different *Bdnf* transcripts to distinct subcellular locations where they may exert different effects on nervous system development. Consistent with this possibility, a recent study found that a sequence contained within the longer 3′ UTR targets *Bdnf* mRNA to distal dendrites. Furthermore, mice that lack the long 3′ UTR have impaired dendritic spine morphogenesis as well as defects in synaptic plasticity (An et al., 2008). Interestingly, of the 18 mRNA transcripts described above, those that are transcribed from promoters I and IV are the most responsive to neuronal activity, raising the possibility that activity may regulate some functions of BDNF but not others (see below).

Unlike genes such as *c-fos* that are induced throughout the body in response to a wide variety of extracellular stimuli, *Bdnf* transcription appears to be largely restricted to the nervous system. This neural specificity of *Bdnf* expression may be accomplished at least in part through the action of the RE1 silencing transcription factor (REST) repressor complex (Chong et al., 1995; Schoenherr and Anderson, 1995; Lunyak et al., 2004). As its name implies, REST binds to neuron restrictive silencer elements (NRSEs or RE1s) and recruits transcriptional repressor complexes, thus silencing the expression of nervous-system-specific genes in nonneuronal cells. *Bdnf* contains an RE1 between its first and second exons, and recent evidence suggests that REST binding to this site represses *Bdnf* transcription in nonneuronal cells (Abuhatzira et al., 2007).

Of the eight *Bdnf* promoters, promoters I and IV are the most responsive to neuronal activity both in vitro (e.g., in cultured neurons in response to membrane depolarization and glutamate release at synapses) and in vivo (e.g., in mice in response to kainate-induced seizure, fear conditioning, and visual stimulation), and most efforts to characterize the mechanisms by which neuronal activity induces *Bdnf* transcription have focused on *Bdnf* promoter IV (referred to as promoter III in earlier literature) (Tao et al., 1998; Timmusk et al., 1993; Rattiner et al., 2004). Initial studies of *Bdnf* transcription employed promoter deletion analysis, DNA-binding studies, biochemistry, and yeast one-hybrid cloning approaches to identify first the DNA regulatory sequences and then the transcription factors that mediate calcium-dependent *Bdnf* promoter IV-driven transcription (Chen et al., 2003b; Tao et al., 1998, 2002). More recently, chromatin immunoprecipitation and bioinformatics studies have helped to expand our understanding of the regulators of activity-dependent *Bdnf* transcription (Chen et al., 2003b; Y. Lin, S. Flavell, and M.E.G., unpublished data). These various approaches have led to the identification of at least six transcription factors (CREB, CaRF, USF, MeCP2, MEF2, and NPAS4) that contribute to activity-dependent *Bdnf* transcription (Chen et al., 2003a; Flavell et al., 2006; Lin et al., 2008) (Figure 3). Prior to neurotransmitter release at synapses, the BDNF promoter is effectively repressed. In this state, *Bdnf* promoter IV is bound by at least four of these transcription factors (CREB, USF, MeCP2, and MEF2). However, despite the presence of multiple transcription factors at BDNF promoter IV, prior to neurotransmitter release and calcium influx into the postsynaptic neuron, the chromatin surrounding the initiation site of *Bdnf* transcription synthesis is bound by histones that are methylated, and the chromatin is condensed, a state that is correlated with gene repression (Chen et al., 2003a; Martinowich et al., 2003). This likely reflects the ability of transcription factors such as MEF2 to recruit histone deacetylases (HDACs), which function to remove activating acetyl groups from histones and histone methyltransferases, which replace the removed acetyl groups with deactivating methyl groups, thus favoring a silenced or repressed state of transcription. In nonneuronal cells, in the absence of calcium signaling to the nucleus, MEF2 interacts with the protein CABIN1, and through this interaction recruits HDACs 1 and 2 as well as the methyltransferase SUV39H1 to repress transcription (Jang et al., 2007; Youn and Liu, 2000; Youn et al., 1999). Although a similar function for CABIN1 has not yet been demonstrated in neurons in the absence of synaptic activity, MEF2 represses transcription in neurons through an interaction with HDACs. Likewise, MeCP2 interacts with the Sin3/HDAC repressor complex, which may further modify the chromatin structure of *Bdnf* promoter IV to inhibit transcription (Nan et al., 1998).

Upon neurotransmitter release at a synapse and the activation of calcium signaling to the nucleus, the transcriptional complex that previously served to repress *Bdnf* promoter IV-dependent transcription is converted to an activating complex and *Bdnf* transcription ensues. The activation of *Bdnf* occurs within several minutes of neurotransmitter release, and *Bdnf* mRNA levels peak 6–8 hr following neuronal stimulation (Hong et al., 2008). For full activation of *Bdnf* transcription to occur, the histones must be demethylated and acetylated. Although it is not fully appreciated how neuronal activity regulates the demethylation of histones at *Bdnf* promoter IV, it has been suggested that the histone demethylase JARID1C/SMCX can demethylate histones bound to the *Bdnf* promoter (Tahiliani et al., 2007). In addition to promoting histone demethylation, neuronal activity also serves to increase histone acetylation at *Bdnf* promoter IV (Tsankova et al., 2004). First, rises in nuclear calcium concentration promote the dissociation of MEF2 from HDACs. This appears to involve the dephosphorylation of MEF2 by calcineurin at serine-408 and the phosphorylation of HDAC4 by CaMKII (Flavell et al., 2006; Zhang et al., 2007). Around the same time, CREB becomes newly phosphorylated at serine-133 (~5 min after

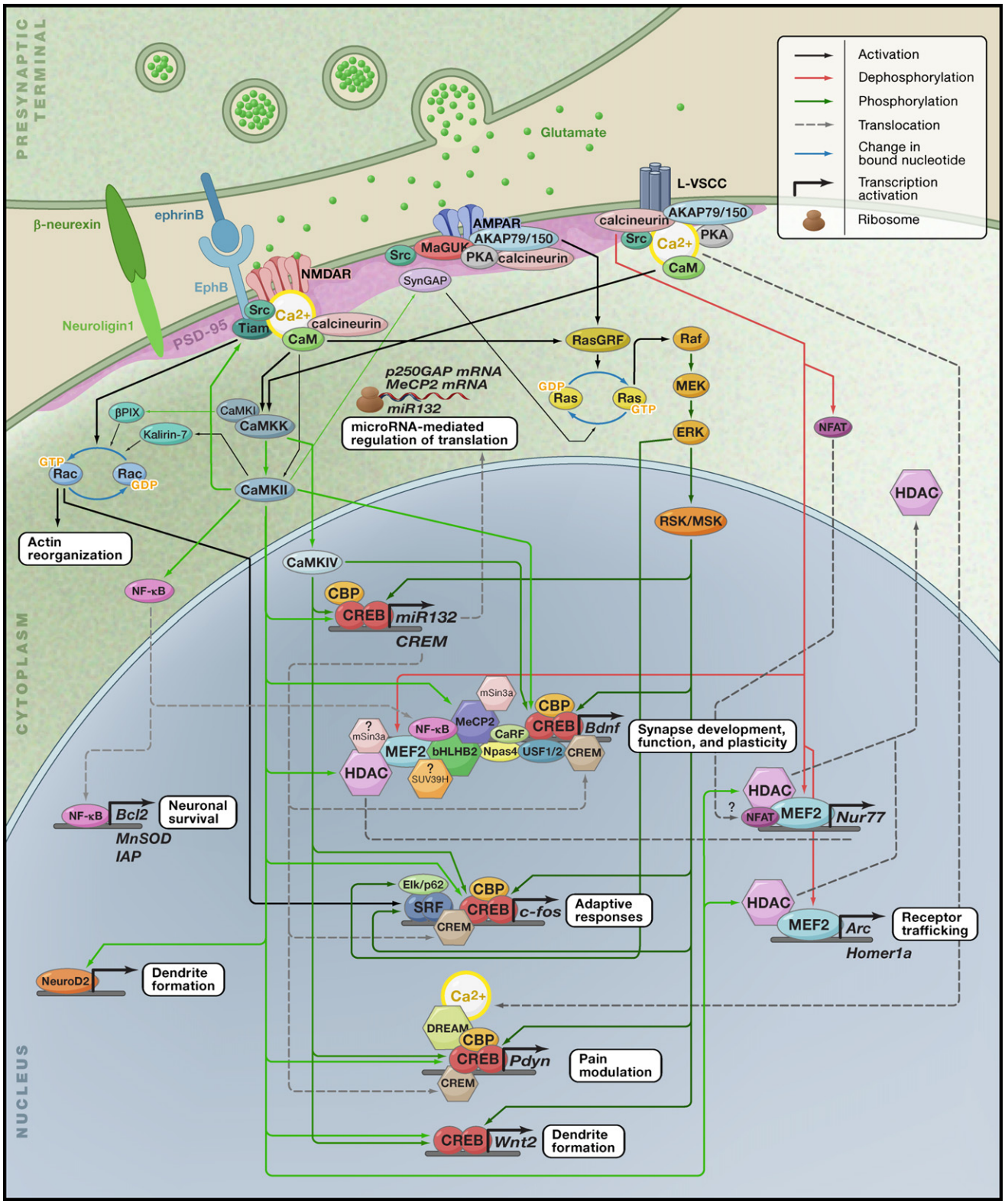

Figure 1. Schematic of Signaling Pathways that Lead to Calcium-Mediated Transcription

Calcium influx through NMDA receptors, AMPA receptors, or L-type VSCCs triggers the activation of a number of signaling molecules that ultimately lead to the expression of new gene products. The signaling cascades induced by calcium influx include the Ras/MAPK pathway, the calcium/calmodulin-dependent protein kinases, the phosphatase calcineurin, and Rac GTPases. These pathways in turn modify the activity of a large number of transcription factors that activate the

neurotransmitter release), which promotes CREB's association with its coactivator, CREB-binding protein (CBP) (Chrivia et al., 1993; Dash et al., 1991; Gonzalez and Montminy, 1989; Sheng et al., 1991). CBP possesses intrinsic histone acetyl transferase (HAT) activity, and likely at least in part through the recruitment of this HAT activity, neuronal activity triggers the acetylation of histones and the recruitment of key components of the RNA polymerase II complex (Bannister and Kouzarides, 1996; Kwok et al., 1994). RNA polymerase II is also likely recruited to *Bdnf* promoter IV in response to calcium influx through the actions of the CREB coactivators, the transducers of regulated CREB activity (TORCs) (Conkright et al., 2003; Iourgenko et al., 2003). Calcium influx induces the dephosphorylation of the TORCs by calcineurin, triggering the translocation of the TORCs into the nucleus (Bittinger et al., 2004). In the nucleus, the TORCs promote the transcription of CREB target genes by recruiting $TAF_{II}130$, a key component of the RNA polymerase holoenzyme.

Following these initial events, a nuclear form of CaMKII appears to phosphorylate MeCP2 at serine-421 ~15–30 min after neurotransmitter release at excitatory synapses (Zhou et al., 2006). While it is not yet clear how phosphorylation of MeCP2 at serine-421 contributes to the activation of BDNF promoter IV transcription, the kinetics of MeCP2 phosphorylation are delayed relative to CREB serine-133 phosphorylation and MEF2 dephosphorylation. The phosphorylation of MeCP2 at serine-421 closely parallels the activation of *Bdnf* transcription, suggesting that MeCP2 serine-421 phosphorylation may be a final step required for *Bdnf* promoter IV transcriptional activation. Consistent with this possibility, the mutation of MeCP2 serine-421 to an alanine partially blocks the neuronal activity-dependent activation of *Bdnf* promoter IV (Zhou et al., 2006).

The observation that MeCP2 is localized to discrete regions of the nucleus containing heterochromatin raises the provocative possibility that MeCP2 may interact with the locus control region (LCR) of DNA (Nan et al., 1996). Locus control regions are transcriptional regulatory sequences that can be present at great distances from the gene that the LCR regulates (Li et al., 2002). LCRs can affect gene expression by a process of DNA looping that brings factors bound to the LCR and the gene promoter in close proximity to one another. It may be that in the unphosporylated state, MeCP2 functions to dock *Bdnf* promoter IV to a repressor locus that ensures that *Bdnf* promoter IV is kept off even though it is in a state ready for transcriptional activation (i.e., transcription factors are already bound to the promoter). If so, then only upon phosphorylation of MeCP2 at serine-421 would *Bdnf* promoter IV be released from this repressor locus, allowing for robust transcription.

Recent evidence suggests that MeCP2, in addition to functioning as a repressor of gene expression, may work as an activator in a complex with CREB (Chahrour et al., 2008). It is possible that MeCP2, CREB, and MEF2 act together to recruit CBP to *Bdnf* promoter IV once CREB and MeCP2 are phosphorylated at serine-133 and serine-421, respectively, and MEF2 is dephosphorylated at serine-408. The binding of CBP to serine-133 phosphorylated CREB may then be the critical step that initiates

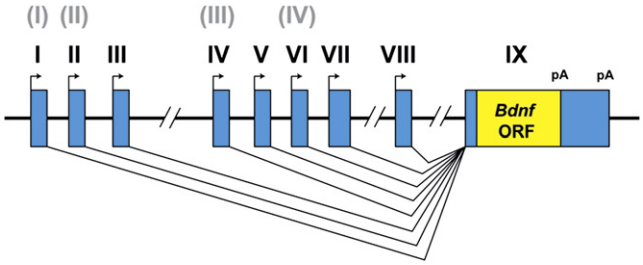

Figure 2. Schematic Representation of the *Bdnf* Gene

Bdnf consists of at least eight distinct promoters (I–VIII) that initiate the transcription of multiple distinct *Bdnf* mRNAs, each of which contains an alternative 5′ exon spliced to a common coding exon (exon IX) that employs either of two polyadenylation sites (pA). As many of these exons have only been recently identified, we have included the old nomenclature for the *Bdnf* exons in gray above for clarity. For example, the current exon IV was previously referred to as exon III.

transcription from *Bdnf* promoter IV. In addition to possessing intrinsic HAT activity, CBP activates transcription by recruiting and stabilizing RNA polymerase II to promoters. Furthermore, CBP is itself phosphorylated on serine-301 by CaMKIV in response to neuronal stimulation, and this phosphorylation activates CBP transcriptional activity (Impey et al., 2002). These observations further suggest a critical role for CBP recruitment in the activation of *Bdnf* promoter IV-dependent transcription.

Once *Bdnf* promoter IV transcription is activated, there appears to be mechanisms in place that function to sustain *Bdnf* transcription when necessary and other mechanisms that ensure that *Bdnf* transcription is shut off at the proper time. Concomitant with *Bdnf* promoter IV activation, a large number of other activity-regulated genes are also induced. One of these activity-regulated genes encodes a transcription factor, NPAS4, that binds to *Bdnf* promoter IV ~30 min after the initial stimulating event and appears to sustain the amount of *Bdnf* mRNA that is transcribed inasmuch as disruption of NPAS4 function leads to an attenuation of *Bdnf* promoter IV transcription (Ooe et al., 2004; Lin et al., 2008). Given recent evidence that Npas4 functions to promote inhibitory synapse formation/function, it is possible that sustaining *Bdnf* transcription may prove to be important for establishing the proper balance between excitation and inhibition during nervous system development.

Within several hours of its activation, *Bdnf* promoter IV transcription returns to the low level that was present in the neuron prior to neurotransmitter release at synapses. Although less is known regarding the mechanism by which *Bdnf* transcription is attenuated, this shut off of *Bdnf* transcription is likely to be important for maintaining the precise levels of BDNF that are required for normal development. The shut off of *Bdnf* transcription is paralleled temporally by the dephosphorylation of MeCP2 and CREB at serine-421 and serine-133, respectively, by phosphatases, including the CREB phosphatase PP1. In addition, MEF2 becomes newly phosphorylated at serine-408, possibly by CDK5 (Gong et al., 2003). Other events that may contribute to the shut off of *Bdnf* promoter IV transcription include the phosphorylation of CREB at serines-142 and -143 (Kornhauser et al., 2002; Gau

transcription of several hundred genes in response to calcium influx. These calcium-responsive genes affect a variety of biological processes, including neuronal survival and dendritogenesis, as well as synaptic development, maturation, and plasticity. This figure is adapted from Zieg et al. (2008).

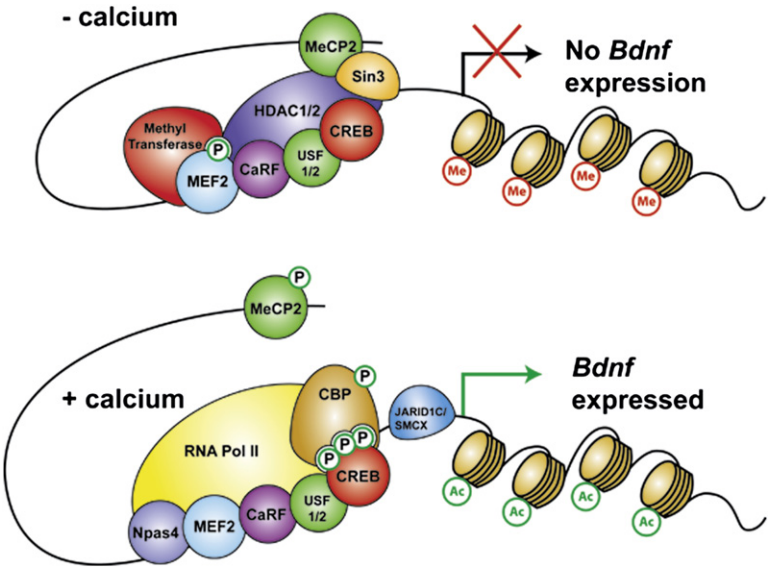

Figure 3. Schematic Representation of *Bdnf* Promoter IV-Bound Transcription Factor Complexes in the Absence and Presence of Calcium Influx

In the absence of calcium influx, *Bdnf* promoter IV is bound by a number of transcription factors (CREB, USF1/2, CaRF, MEF2, and MeCP2) that function to recruit repressor complexes, including HDACs and methyltransferases. Upon calcium influx, these transcription factors are modified, as is the state of the chromatin surrounding *Bdnf* exon IV. These modifications include the phosphorylation of MeCP2 and CREB and the dephosphorylation of MEF2. NPAS4 is also newly transcribed and translated and binds to the proximal promoter of *BDNF* exon IV. These modifications result in the recruitment of the transcriptional coactivator CBP and the RNA polymerase II holoenzyme, which in turn leads to the transcription of *BDNF* exon IV.

et al., 2002). Phosphorylation at these sites occurs with a delayed time course relative to the phosphorylation of CREB at serine-133. Phosphorylation of CREB at serines-142 and -143 inhibits CREB binding to CBP, and, consistent with the idea that phosphorylation of these sites could inhibit CREB activity, mutation of serine-142 to the nonphosphorylateable alanine leads to the enhanced activation of CREB target genes. These results raise the possibility that phosphorylation of CREB on serines-142 and -143 may be part of the mechanism for shutting off *Bdnf* transcription.

It is clear from the work described above that there is a large amount now known about the mechanisms by which neuronal activity regulates *Bdnf* transcription. However, despite the progress in identifying the repressor and activator complexes that control activity-dependent *Bdnf* promoter IV transcription, much remains to be determined. We still do not understand the roles of CaRF or USF in regulating transcription from the *Bdnf* promoter, and there are a number of calcium-dependent modifications that occur to the transcription factors that regulate *Bdnf* to which a function and/or mechanism have yet to be ascribed (e.g., the acetylation/sumoylation of MEF2, the phosphorylation of MeCP2, and the phosphorylation of CREB at serines-142 and -143).

Furthermore, much of the work described above has been carried out in cultured neurons, and additional experiments will be required to determine the role of the various calcium-regulated transcription factors in the control of *Bdnf* transcription in response to physiological stimuli in vivo. To date, experiments that have been performed in vivo have yielded results that are similar to those using cultured neurons, suggesting that the observations described above are likely to hold true under more physiological conditions. For example, *Bdnf* promoter I and IV are the most highly induced of the eight *Bdnf* promoters in response to physiological in vivo stimuli (e.g., fear conditioning and visual stimulation) (Rattiner et al., 2004; Hong et al., 2008). In addition, enhancing or blocking the function of transcription factors that regulate the transcription of *Bdnf* promoter IV in vitro has been shown to have similar effects in vitro and in vivo. This has been best demonstrated for CREB, where expression of a constitutively active form of CREB (CREB-VP-16) in mice drives the transcription of *Bdnf* (Barco et al., 2005). Conversely, a subtle mutation of the CREB-binding site in *Bdnf* promoter IV in mice significantly blocks the induction of *Bdnf* by physiological stimuli such as visual experience (Hong et al., 2008). These results suggest that the conclusions drawn from in vitro studies of the mechanisms of activity-dependent gene regulation are likely to be highly relevant in vivo.

Perhaps the most significant remaining question is why there are so many factors that contribute to the regulation of the transcription of this one gene in response to calcium influx into neurons. There are a number of possible reasons why the nervous system has evolved such intricate mechanisms to regulate *Bdnf* transcription. The most obvious possibility is that this allows for a precise temporal and scaled response ensuring that exactly the right amount of BDNF is expressed at the appropriate time and place. By activating or not activating a given transcription factor, the neuron may be able to dial up or down the amount of *Bdnf* mRNA that is transcribed. For example, the presence of multiple transcription factors may allow the neuron to respond differently to different frequencies of stimulation and levels of cytoplasmic and nuclear calcium. It is also possible that different cell types express unique combinations of the transcription factors that regulate *Bdnf* transcription, thus facilitating differential BDNF responses in different cell types. Finally, the large number of transcription factors that regulate *Bdnf* transcription may simply reflect the fact that *Bdnf* transcription is complex—that in the absence of activity *Bdnf* is repressed and that following stimulation *Bdnf* is turned on transiently before it is again shut off. It may be that a large number of factors are required to effectively accomplish each of these tasks.

Other Activity-Regulated Genes in Nervous System Development

Although we have focused this review on the regulation of *Bdnf* promoter IV transcription, the mechanisms that control *Bdnf* promoter IV transcription are likely to also be relevant to the regulation of many other activity-regulated genes. As discussed above, several hundred genes have been identified whose transcription is regulated by neuronal activity. Genome-wide analyses of

transcription factor binding sites have revealed that CREB, MEF2, and Npas4 are likely to control the activity-dependent transcription of a large number of activity-regulated genes in addition to *Bdnf* (Flavell et al., 2006; Impey et al., 2004). However, these three transcription factors by themselves do not account for the entirety of activity-dependent gene transcription. In fact, there are a large number of additional transcription factors that mediate neuronal-activity-driven transcription, including SRF, ELK, NFAT, NFkB, DREAM, NeuroD, SP4, and CREST, and for each of these factors the mechanism of activation in response to calcium influx appears to be distinct (Aizawa et al., 2004; Carrion et al., 1999; Gaudilliere et al., 2004; Graef et al., 1999; Meffert et al., 2003; Ramos et al., 2007; Shaw et al., 1989; Treisman, 1987). For instance, SRF and NeuroD are phosphorylated and activated in the nucleus by the calcium-activated RSK/MSK and CaMKII kinases, respectively (Gaudilliere et al., 2004; Rivera et al., 1993). On the other hand, NFAT and NFkB are localized to the cytoplasm prior to stimulation. Calcium influx into the neuron triggers the dephosphorylation of NFAT by calcineurin and the phosphorylation of NF-kB by CaMKII, which in turn lead to the translocation of NFAT and NF-kB to the nucleus where they activate transcription (Graef et al., 1999; Meffert et al., 2003). The mechanism of downstream regulatory element-antagonist modulator (DREAM) activation is also distinct. Prior to neuronal stimulation, DREAM binds to the promoters of its target genes and represses their transcription. Upon neurotransmitter release, DREAM binds directly to calcium ions that enter the nucleus, and upon binding to calcium, DREAM is released from the promoters of its target genes, thus relieving transcriptional repression and allowing transcription of these genes (Carrion et al., 1999).

Interestingly, these activity-regulated transcription factors control the expression of overlapping but distinct subsets of activity-regulated genes, suggesting a mixing and matching of transcription factors to achieve the precise temporal, spatial, and stimulus-specific control as well as the appropriate magnitude of induction for each of the activity-regulated genes that is needed for proper synaptic development. The large number of genes that are regulated by neuronal activity as well as the precise control of the expression of these genes in response to neurotransmitter release suggest that the program of activity-dependent gene expression is likely to play an important role in nervous system function. Consistent with this hypothesis, components of the activity-dependent transcription program have been shown to play key roles in many different aspects of nervous system development, including neuronal survival, dendritic growth, synapse formation, synapse refinement, and synaptic plasticity. As the role of activity-dependent transcription in neuronal survival has been reviewed (Mattson and Meffert, 2006; Bonni and Greenberg, 1997), we will focus here on how activity-dependent transcription regulates synapse development and function.

Activity-Dependent Gene Regulation and Biology

Dendritic Development

In the CNS, the vast majority of synapses form onto the dendrites of neurons. As the dendritic structure of the neuron is a crucial determinant of the processing of synaptic inputs, one way in which neuronal activity regulates synaptic processing is by altering the length or complexity of dendrites. Dendritic growth is a highly dynamic process that is tightly regulated by synaptic activity so that appropriate connections are formed and maintained. Sensory blockade by monocular deprivation significantly alters dendritic development in both the lateral geniculate nucleus and the visual cortex (Coleman and Riesen, 1968; Wiesel and Hubel, 1963). These observations suggested that neuronal activity plays a critical role in dendritic development and have been extended by a large number of more recent studies (Chen and Ghosh, 2005; Cline, 2001; Hooks and Chen, 2007). For instance, blocking neuronal activity in vivo by infusion of the sodium channel blocker tetrodotoxin (TTX) results in reduced dendritic branching of CA1 hippocampal pyramidal neurons (Groc et al., 2002). Conversely, exposure of rodents to an enriched environment leads to an increase in dendritic growth and an elaboration of dendritic arborization (Faherty et al., 2003). Likewise, in *Xenopus* tectum, light-induced visual activity increases the growth of new dendritic branches and concurrently stabilizes existing branches (Sin et al., 2002). Taken together, these results and others suggest a broad role for activity in promoting dendritic growth and maintenance.

Activity-dependent gene transcription plays a key role in mediating the effect of neuronal activity on dendritic growth. Disrupting CREB function interferes with the ability of neuronal activity to influence dendritic growth (Redmond et al., 2002; Wayman et al., 2006). Similarly, blocking NeuroD function by RNAi or preventing the activity-dependent phosphorylation of NeuroD on serine-336 is sufficient to block calcium-mediated dendritic growth (Gaudilliere et al., 2004). Another activity-regulated transcription factor that plays a role in regulating dendritic growth is the calcium-responsive transactivator, CREST. Layer V cortical and CA3 pyramidal neurons of CREST knockout mice have significantly fewer dendrites and are not responsive to membrane-depolarizing stimuli that induce the growth of dendrites in wild-type neurons (Aizawa et al., 2004).

In addition to the effects on dendritic growth of the activity-regulated transcription factors described above, a number of genes whose transcription is regulated by neuronal activity also play an important role in controlling dendritic growth. A screen for potential CREB target genes identified the micro RNA miRNA132 as a CREB target (Impey et al., 2004). Overexpression of miRNA132 increases, while blocking miRNA132 function decreases, neurite growth (Vo et al., 2005). miRNA132 controls dendritic growth by regulating the translation of p250GAP, a protein that affects actin cytoskeletal dynamics (Wayman et al., 2008a). Similarly, overexpression of candidate plasticity gene 15 (cpg15) enhances dendritic outgrowth as well as axonal elaboration (Cantallops et al., 2000; Nedivi et al., 1998). Together, these results suggest a broad role for activity-dependent gene induction in regulating synaptic-activity-driven dendritic remodeling.

Synapse Elimination

Often during the development of neuronal circuits, a larger number of synapses are formed than are ultimately present within the mature neural circuit, and these excess synapses are eliminated in a manner that is dependent upon sensory-experience-driven synaptic activity (Katz and Shatz, 1996; Sanes and Lichtman, 1999). The role of synaptic activity in synapse elimination has been particularly well characterized at the neuromuscular

junction (NMJ). Initially, individual muscle fibers are innervated by multiple motor neuron axons. However, during early postnatal development, synapse elimination occurs until the muscle fiber is innervated by a single motor neuron axon. This elimination occurs by a process where a single input gradually becomes stronger, and the other inputs become weaker before being eliminated (Colman et al., 1997). Blocking synaptic activity by inhibiting neurotransmitter release prevents synapse elimination at the NMJ, and as a result, muscle fibers remain multiply innervated. A similar process of synapse elimination occurs in the CNS (e.g., the retinogeniculate synapse, the synapse made by retinal ganglion neurons onto thalamic neurons, and the climbing fiber/Purkinje cell synapse in the cerebellum) (Chen and Regehr, 2000; Nishiyama and Linden, 2004). These results suggest a broad and important role for neuronal activity in refining synaptic connectivity so that appropriate synaptic connections are maintained and inappropriate synapses are eliminated.

It has recently been found that several of the components of the activity-dependent transcription network control synapse number and thus could be regulators of the process of synapse elimination. Interfering with MEF2 function results in an increase, while overexpression of MEF2 results in a decrease, in the number of excitatory synapses that form on hippocampal neurons (Barbosa et al., 2008; Flavell et al., 2006). The ability of MEF2 to restrict synapse number is dependent on calcium and calcineurin and the ability of MEF2 to mediate activity-dependent transcription, suggesting that MEF2 target genes are likely to play important roles in the regulation of synapse number. *Homer1a* and *Arc* are activity-regulated MEF2 target genes that may mediate MEF2's effect on synapse number. Induction of Homer1a interferes with the assembly of synaptic complexes, thus serving to reduce synapse number, while increasing Arc levels leads to the internalization of AMPA receptors at synapses, thus weakening the synapse and rendering it more prone to elimination (Chowdhury et al., 2006; Rial Verde et al., 2006; Sala et al., 2003).

Another activity-regulated gene that functions to restrict synapse number is the serum-inducible kinase (*SNK*). Once *SNK* expression is induced by synaptic activity, SNK phosphorylates SPAR, a postsynaptic scaffolding molecule that functions to promote synapse growth. However, following phosphorylation by SNK, SPAR is rapidly degraded by the ubiquitin-proteasome complex, resulting in a destabilization of synapses (Pak and Sheng, 2003). Thus, activity-dependent *SNK* induction leads to SPAR degradation, resulting in a decrease in synapse number. Whether SNK and/or MEF2 and its target genes (*homer1A* and *Arc* and others) play a direct role in developmental synapse elimination in vivo and/or are involved in homeostatic control of synapse number in response to excess excitation within a neural circuit remains to be determined.

Excitatory/Inhibitory Balance

The proper balance of excitatory and inhibitory synaptic input is crucial for processing sensory information as well as for higher cognitive functions, and an increasing number of human neurological disorders are characterized by imbalances in excitatory and inhibitory synaptic strength. Thus, there is considerable interest in defining the mechanism by which neuronal activity regulates inhibitory synapse development and maturation (Cline, 2005). Several recent studies have revealed that, in addition to the ability to regulate the number of excitatory synapses, components of the activity-dependent gene network also appear to control the development and/or maintenance of inhibitory synapses. Recent work identified a transcription factor, NPAS4, that may control the balance between synaptic excitation and inhibition within the CNS (Lin et al., 2008).

NPAS4 is a bHLH-PAS domain transcription factor whose transcription is rapidly and transiently induced following calcium influx into neurons. NPAS4 controls the number of inhibitory synapses that form on excitatory neurons. The inhibition of NPAS4 expression leads to a reduction, while overexpression of NPAS4 leads to an increase, in the number of inhibitory synapses that form on excitatory neurons. The acute deletion of NPAS4 in hippocampal neurons within an intact neural circuit in slice culture results in a significant reduction in mEPSC frequency. This suggests that NPAS4 also controls excitatory synaptic function, possibly as an indirect consequence of the effect of loss of NPAS4 on inhibitory synapse number. These results indicate that NPAS4 functions to increase synaptic inhibition and decrease excitation, suggesting that NPAS4 is critical for maintaining an appropriate balance of excitation and inhibition. In addition to NPAS4, MEF2 may also play a role in the control of excitatory/inhibitory balance through its ability to regulate excitatory synapse number.

The balance between synaptic excitation and inhibition can be altered by changing the strength as well as the number of excitatory and/or inhibitory synapses. Recently, the activity-regulated transcriptional regulator MeCP2 has been shown to specifically control the strength of excitatory synaptic connections (Dani et al., 2005). Layer 5 cortical pyramidal neurons from MeCP2 knockout mice have significantly reduced spontaneous synaptic activity due to a reduction in mEPSC amplitude. However, there is no corresponding change in mIPSC amplitude in these mice, suggesting that the alteration in excitability in MeCP2 knockout mice is due to a change in the balance of excitatory and inhibitory synaptic strength.

It is possible that the effects of NPAS4, MEF2, and MeCP2 on the balance between neuronal excitation and inhibition may be explained at least in part by the ability of these factors to regulate *Bdnf* promoter IV transcription. A recent study provides some support for the idea that activity-dependent transcription of *Bdnf* controls excitatory/inhibitory balance (Hong et al., 2008). Mice were generated in which a subtle change was introduced within the CREB-binding site of *Bdnf* promoter IV that precludes CREB binding. These knockin mice express similar levels of *Bdnf* in the absence of stimulation but have significantly reduced levels of *Bdnf* promoter IV-dependent mRNA transcripts following synaptic stimulation. Notably, in these knockin mice, significantly fewer inhibitory synapses form on excitatory neurons, suggesting that activity-dependent *Bdnf* transcription plays a key role in regulating the number of inhibitory synapses.

Synaptic Plasticity

Sensory experience also affects neuronal connectivity within the adult brain, and there is evidence that such neuronal plasticity is mediated at least in part by the activity-regulated gene program. This is true in paradigms of associative learning such as when an electrical shock to the foot of an animal is delivered shortly after

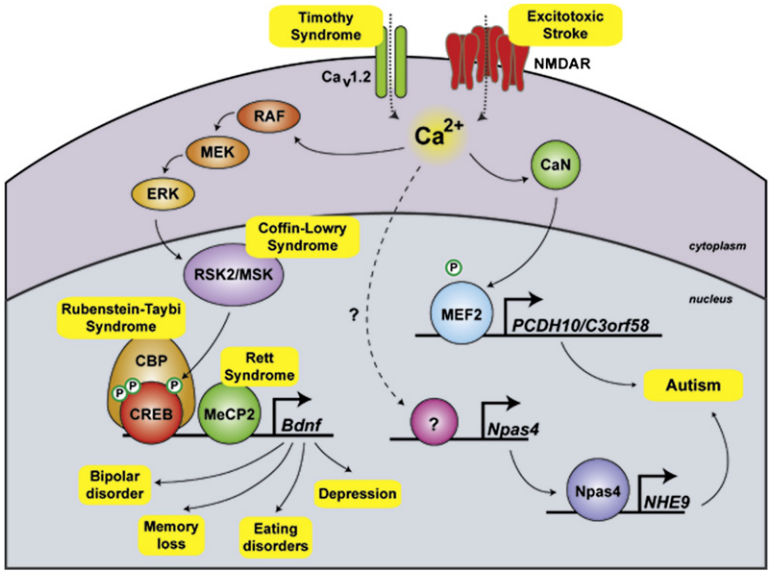

Figure 4. Mutation of Components of the Activity-Dependent Gene Expression Program Results in Human Cognitive Disorders

Mutation of a number of molecules that regulate *Bdnf* transcription results in human cognitive disorders. These include $Ca_V1.2$ (Timothy syndrome), RSK2 (Coffin-Lowry syndrome), CBP (Rubenstein-Taybi syndrome), MeCP2 (Rett syndrome), and BDNF itself (memory loss and psychiatric disorders). The mutation of components of other activity-dependent gene expression pathways lead to additional cognitive disorders, including especially autism.

an auditory stimulus is administered. After several repetitions of this paired stimulus, animals learn that the auditory stimulus is linked to the shock and exhibit a fear response when presented with the auditory stimulus even in the absence of electrical shock. This learning is accomplished by altering the strength of synaptic connections within the dorsolateral nucleus of the amygdala, which receive direct input from the auditory system and are required for this type of associative learning. The neurons of the dorsolateral nucleus significantly increase their firing rate in response to the auditory stimulus once associative fear conditioning has occurred (Maren and Quirk, 2004). These changes in firing rate share many similarities with long-term potentiation (LTP), an activity-dependent model for synaptic strengthening, which is characterized by the insertion of AMPA receptors into the postsynaptic membrane and the formation of new synapses (Engert and Bonhoeffer, 1999; Malinow and Malenka, 2002). Both associative learning and long-lasting forms of LTP are correlated with, and have been shown to be dependent upon, activity-dependent gene transcription.

The initial observation that activity-dependent transcription is required for associative learning was made by Kandel and colleagues in the snail *Aplysia californica*. *Aplysia* undergo a form of associative learning called sensitization in which the repeated electrical stimulation of the snail's tail triggers a defensive gill-withdrawal response (Kandel, 2001). The *Aplysia* homolog of CREB, CREB-1, is induced in sensory neurons following electrical stimulation, and the inhibition of CREB-1 function interferes with the strengthening of sensory neuron connections required for sensitization (Dash et al., 1990; Kaang et al., 1993). These experiments suggest that activity-dependent gene regulation might be relevant to synaptic plasticity and learning. Experiments in mammals have provided support for this possibility. Targeted deletion of CREB in mice results in a defect in LTP induction at Schaffer collateral-CA1 synapses in the hippocampus, while regulated expression of a constitutively active form of CREB (CREB-VP16) results in enhanced LTP (Bourtchuladze et al., 1994; Barco et al., 2002). A stimulation paradigm that in wild-type mice results in only a short-lasting form of LTP elicits a longer-lasting form of LTP in CREB-VP-16 mice (Barco et al., 2002). However, since CREB plays a critical role in regulating both neuronal surival and dendritic outgrowth, it is difficult to determine from these experiments whether CREB's effect on plasticity is secondary to an effect of CREB on dendritic growth and/or apoptosis. However, additional evidence in support of a role for activity-dependent transcription in synaptic plasticity comes from a study of mice that carry a conditional targeted deletion of the activity-regulated transcription factor SRF, which reveals that SRF-deficient mice have defects in LTP at Schaffer collateral-CA1 synapses but no impairment in cell survival (Etkin et al., 2006; Ramanan et al., 2005). Taken together, the results described above suggest a critical role for activity-dependent transcription in experience-driven synaptic development and function.

Activity-Dependent Transcription and Human Cognition

The symptoms of many disorders of human cognition first present during early postnatal development, the time during which activity-dependent genes are highly induced and experience-dependent synaptic remodeling is peaking. These observations raise the possibility that defects in the activity-dependent gene program could account for some disorders of human cognition. Consistent with this hypothesis, accumulating evidence has indicated that mutations in a large number of the components of the activity-dependent gene program give rise to disorders of human cognition (Figure 4).

BDNF

A polymorphism in the coding region of the human *Bdnf* gene (Val66Met) has a profound impact on hippocampal-dependent memory (Egan et al., 2003; Hariri et al., 2003). Individuals with this polymorphism not only perform less well in tests of learning and memory but also have increased susceptibility to a number of neuropyschiatric disorders, including depression, eating disorders, and bipolar disorder (Neves-Pereira et al., 2002; Ribases et al., 2003, 2004; Sen et al., 2003; Sklar et al., 2002).

Given that polymorphisms in the *Bdnf* gene are associated with impairments in human cognition, it has been hypothesized that the mutation of components of the pathways that control activity-dependent *Bdnf* promoter IV transcription might also result in disorders of human cognition. A number of recent studies have provided significant support for this hypothesis. Mutation of a subtype of the L-type VSCC (Ca_V-1.2) is the cause of Timothy syndrome, a human disorder that results in significant cognitive

impairment and a high prevalence of autism spectrum disorders (~80%), suggesting a critical role for the Ca_v-1.2 gene in human cognitive development (Splawski et al., 2004). Likewise, mutation of the CREB cofactor CBP results in Rubinstein-Taybi syndrome, a disorder characterized by severe mental retardation (Petrij et al., 1995). CBP is important for activity-dependent transcription, as it associates with CREB only after CREB has been phosphorylated at serine-133 and, upon its association with CREB, increases the transcription of *Bdnf*. One of the kinases that phosphorylates CREB on serine-133, RSK2, is mutated in Coffin-Lowry syndrome, another human disorder that results in severe mental retardation (Hanauer and Young, 2002). Finally, mutation of MeCP2, an activity-regulated repressor of BDNF promoter IV transcription, results in Rett syndrome, a pervasive developmental disorder characterized by mental retardation and defects in socialization. A striking feature of Rett syndrome is that this disorder is first detected within the second year of life following apparently normal early development. This is the time when activity-dependent synaptic development is underway (Zoghbi, 2003) and raises the possibility that Rett syndrome is a disorder of activity-dependent gene transcription. Several features of MeCP2 make this hypothesis compelling. These include the observations that calcium-dependent phosphorylation of MeCP2 affects dendritic spine development and loss of MeCP2 function alters excitatory/inhibitory synaptic balance (Dani et al., 2005; Zhou et al., 2006). As Rett syndrome is an autism spectrum disorder, these findings on MeCP2 provide the first compelling evidence that defects in activity-dependent gene transcription might be a cause of autism.

Other Activity-Dependent Transcription Factors and Human Cognitive Disorders

If the hypothesis that mutation of genes involved in the activity-dependent gene regulation program results in human cognitive disorders is correct, then one would expect that mutation of pathways in addition to the BDNF pathway would also result in disorders of human cognition, including autism. A recent study aimed at identifying genetic loci that are mutated in autism identified several activity-regulated genes as autism loci. Genetic analysis of individuals with autism derived from consanguineous lineages identified two large genomic deletions that cosegregated with autism (Morrow et al., 2008). Strikingly, the deletions are not within the coding regions of genes but rather appear to include sequences that control gene expression. The three genes closest to these deletions are *c3orf58*, *NHE9*, and *PCDH10*, suggesting that these deletions may cause autism by interfering with the regulation or function of these genes. *c3orf58* is a gene of unknown function, while *PCDH10* encodes a member of the protocadherin family, which have been implicated in synapse development, and *NHE9* encodes for a sodium/proton pump that is important for maintaining the proper balance of ions within neurons. Interestingly, *c3orf58*, *NHE9*, and *PCDH10* appear to be activity-regulated genes (Morrow et al., 2008). *c3orf58* mRNA is induced by membrane depolarization of neurons, contains evolutionarily conserved MEF2, CREB, and SRF binding sites, and its activity-induced transcription is significantly reduced by interfering with MEF2 function. Similarly, *PCDH10* mRNA is induced by membrane depolarization, and its expression is driven by an activated form of MEF2. Finally, *NHE9* expression is regulated, at least in part, by the action of the activity-regulated transcription factor NPAS4.

The observation that the mutations associated with *NHE9* and *PCDH10* that were identified in autistic individuals are not located within the coding region of these genes suggests that these mutations instead are likely to affect regulatory regions that control the level of gene transcription of *NHE9* and *PCDH10*. Thus, these mutations might be expected to not completely knock out gene function, and this could explain why the mutations identified in *NHE9* and *PCDH10* result in autism and epilepsy and not a more significant mental retardation. It is interesting to speculate that autism and epilepsy may arise from mutations that affect the regulation of genes involved in synapse development and/or function, while mutations that disrupt the coding region of genes may cause more severe phenotypes such as mental retardation. These observations suggest that as more mutations within the regulatory regions of activity-dependent genes are discovered they may be found to disproportionately give rise to autism spectrum disorders and epilepsy as opposed to mental retardation that results from a loss of gene function.

Concluding Remarks

As described in this review, activity-regulated transcription plays a crucial role in many different aspects of synaptic development, and thus a significant amount of effort has gone into elucidating the molecular pathways that regulate activity-dependent transcription. As more of the components of the signaling network that controls activity-dependent gene transcription are identified, and the causal mutations of human cognitive disorders become known, it is striking that there is a large degree of overlap between the regulators of activity-dependent gene expression and genes that are mutated in human cognitive disorders.

Nevertheless, we are currently limited by what we know about human cognitive disorders as well as by our current understanding of the activity-dependent gene regulation program. The work described above on the activity-dependent transcriptional network and the corresponding human cognitive disorders suggests that extensive characterization of signaling pathways as well as human mutations has the potential to greatly increase our understanding of the link between these processes. To this end, advances in sequencing technology allowing for fast, inexpensive, complete sequencing of DNA and RNA in mammalian cells suggest that the next few years will see an explosion in the identification of mutations associated with human cognitive disorders. Similarly, the ability to assess chromatin occupancy of transcription factors on a genome-wide scale and to use RNAi to disrupt gene function suggests that the identification of the targets of activity-regulated transcription factors will not be far behind. Taken together, these approaches will allow for a more thorough comparison of the genes involved in disorders of human cognition and the components of the activity-regulated gene network. We believe that in the years ahead these avenues of research will greatly increase our understanding of nervous system development and also shed light on the etiology of many disorders of human cognition.

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